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(54) STRESS PROTEINS AND USES THEREFOR

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PROTEINES DU STRESS ET LEURS UTILISATIONS

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- **EUROPEAN JOURNAL OF IMMUNOLOGY vol. 22, no. 6 , June 1992 , WEINHEIM, DE pages 1365 - 1372 C. BARRIOS ET AL. 'Mycobacterial heat-shock proteins as carrier molecules. II: The use of the 70-kDa mycobacterial heat-shock protein as carrier for conjugated vaccines can circumvent the need for adjuvants and Bacillus Calmette Guérin priming' cited in the application**
- **DINTZIS ET AL. : "", PEDIATRIC RESEARCH, , 1992, vol. 32, no. , pages 376 to 385**
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Description

Background of the Invention

[0001] Although the function of stress proteins is not entirely clear, it appears that some particpate in assembly and structural stabilization of certain cellular and viral proteins, and their presence at high concentrations may have an additional stabilizing effect during exposure to adverse conditions. Neidhardt, F.C. and R.A. Van Bogelen, In: Escherichia coli and Salmonella typhimurium, Cellular and Molecular Biology, (eds. Neidhardt, F.C., Ingraham, J.L., Low, K.B., Magasanik, B. Schaechter, M. and Umbarger, H.E. (Am. Soc. Microbiol., Washington, D.C.), pp. 1334-1345 (1987); Pelham, H.R.B. Cell, 46:959-961 (1986); Takano, T. and T. Kakefuda, Nature, 239:34-37 (1972); Georgopoulos, C. et al., New Biology, 239:38-41 (1972). Phagocytic host cells produce a hostile environment for foreign organisms, and the ability to produce stress proteins has been implicated in the survival of bacterial pathogens within macrophages Christman, M.F. et al., Cell, 41:753-762 (1985).

[0002] Mycobacterium (M.) tuberculosis and Mycobacterium (M.) leprae are the etiologic agents of tubercolosis and leprosy, respectively. These diseases afflict 20-30 million people and continue to present a significant global health problem. Joint International Union Against Tubercolosis and World Health Organization Study Group, Tubercle, 63: 157-169 (1982); Bloom, B. and T. Godal, Rev. Infect Dis. 5:765-780 (1983). To develop more effective tools for the diagnosis and prevention of these diseases, it is important to understand the immune response to infection by mycobacterial pathogens.

[0003] The antibody and T-cell responses to infection or inoculation with killed mycobacteria have been studied in humans and in animals. Human patients with tuberculosis or leprosy produce serum antibodies directed against at least 12 mycobacterial proteins. Some of these proteins are also recognized by well-characterized murine monoclonal antibodies. Mice immunized with mycobacterial lysates produce antibodies that are directed predominantly to six M. tuberculosis and six M. leprae protein antigens. Engers, H.D. Infect. Immun., 48:603-605 (1985); Engers, H.D., Infect. Immun., 51:718-720 (1986). Genes encoding these 12 mycobacterial antigens have been cloned, and recombinant proteins produced from these clones have been used to investigate the human T-lymphocyte response to mycobacterial infection. Husson, R.N. and R.A. Young, Proc. Natl. Acad. Sci., USA, 84:1679-1683 (1987); Young, R.A. et al., Nature, 316:450-452 (1985); Britton, W.J. et al., Lepr. Rev., 57, Suppl. 2, 67-75 (1986).

[0004] Protection against mycobacterial disease involves cell-mediated immunity. Joint International Union Against Tuberculosis and World Health Organization Study Group, Tubercle, 63:157-169 (1982); Hahn, H. and S.H.E. Kaufman, Rev. Infect. Dis., 3:1221-1250 (1981). T-lymphocytes cloned from patients or from volunteers immunized with killed mycobacteria have been tested for their ability to recognize the recombinant mycobacterial proteins. Lymphocyte-proliferation assays demonstrate that most of the antigens identified with monoclonal antibodies are involved in the T-cell response to mycobacterial infection or vaccination in mice and in humans. Limiting dilution analysis indicates that 20% of the mycobacterial-reactive $CD4^+$ T-lymphocytes in mice immunized with M. tuberculosis recognize a single protein, the 65-kDa antigen. Kaufman, S.H.E. et al., Eur J. Immunol., 17:351-357 (1987).

Summary of the Invention

[0005] The present invention relates to the subject matter of the claims. The invention finds application in immune therapy or prophylaxis, which results in an induction or enhancement of an individual's immune response and as an immunotherapeutic agent which results in a decrease of an individual's response to his or her own cells. In the embodiment in which an individual's immune response is induced or enhanced, the induced or enhanced response can be a response to antigens, such as those derived from a pathogen or cander cell, or can be upregulation of the individual'a immune status, such as in an immune compromised individual. In immune prophylaxis, effects in an individual of a pathogen, which can be any virus, microorganism, parasite or other organism or substance (e.g., a toxin or toxoid) which causes disease or the effects in an individual of cancer cells, are prevented or reduced. In preventing or reducing adverse effects of pathogens which contain stress proteins (e.g., bacteria, parasite, fungus) an individual's immune response to the pathogen's stress protein(s) is induced or enhanced. The stress protein is administered joined to another antigen by recombinant means lie joined to a fusion partner resulting in a fusion protein.

[0006] Preventing or reducing adverse effects of viral pathogens which do or do not contain stress proteins, as well as preventing or reducing the adverse effects of cancer cells according to the present method, is effected by enhancing an individual's immune surveillance system. Enhancement of immune response can be effected by modulating the immune cells by stimulation with a fusion protein of the invention (e. g., comprising a bacterial stress protein).

[0007] Where an individual's immune response is decreased, such as is used in treating autoimmune diseases, fusion proteins comprising stress proteins known to be involved in the autoimmune response are administered to turn down an individual's immune response by tolerizing the individual to the stress proteins. Alternatively, the immune response to stress protein, which is known to occur in autoimmune disease, is reduced by interfering with the ability

of immune cells which respond to stress proteins to do so.

[0008] A fusion protein of the present invention can be administered to an individual, and result in an immune response which provides protection against subsequent infection by a pathogen (e.g., bacteria, other infectious agents which produce stress proteins) or reduction or prevention of adverse effects of cancer cells. Alternatively, a fusion protein can be administered to an individual, generally over time, to induce immune tolerance against the selected stress protein. For example, a fusion protein can be administered in multiple doses over time in order to induce immune tolerance against an autoimmune disease such as rheumatoid arthritis.

Brief Description of the Drawings

[0009] Figure 1 is a graphic representation of the homologies between mycobacterial antigens and known stress proteins. Figure 1A is a representation of sequence similarity between portions of the M. tuberculosis 71-kDa antigen (residues 1-204; TB 71 kDa) and the E. coli DnaK protein (residues 430-639). Figure 1B is a representation of sequence similarity between portions of the M. tuberculosis 65-kDa antigen (residues 1-540; TB 65 kDa) and the E. coli GroEL protein (residues 1-547).

[0010] Figure 2 is a comparison of the amino acid sequence of the human P1 protein (573 residues) (SEQ ID NO: 1) and the amino acid sequence of the groEL protein (547 residues) (SEQ ID NO: 2).

[0011] Figure 3 is a comparison of the amino acid sequence of the human P1 protein (573 residues) (SEQ ID NO: 1), which is a homolog of groEL protein, and the amino acid sequence of the 65 kDa M. leprae protein (540 residues) (SEQ ID NO: 3).

[0012] Figure 4 is a comparison of the amino acid sequence of the human P1 protein (573 residues) (SEQ ID NO: 1), which is a homolog of the groEL protein, and the amino acid sequence of the 65kDa M. tuberculosis protein (540 residues) (SEQ ID NO: 4).

[0013] Figure 5 is a schematic representation of selected stress protein fusion vectors which contain a polylinker with multiple cloning sites permitting incorporation of a gene of interest.

[0014] Figure 6 is a schematic representation of the stress protein fusion vector, pKS70 containing the T7 RNA polymerase promoter, a polylinker and the *mycobacterial tuberculosis* hsp70 gene, and the stress protein fusion vector pKS72 containing the HIV p24 gag gene subcloned into the pKS70 vector.

[0015] Figure 7 is a graph illustrating the anti-p24 antibody titer in mice injected with the p24-hsp70 fusion protein, p24 alone and hsp70 alone.

Detailed Description of the Invention

[0016] Cells respond to a variety of stressful stimuli by increasing the synthesis of specific stress proteins. The most extensively studied cellular response to stressful stimuli is the synthesis of heat shock proteins (hsp) by a cell, induced by a sudden increase in temperature. Because many of the heat shock proteins are also induced by other stresses, they are frequently called stress proteins. Stress proteins and their relatives appear to help assemble and disassemble protein complexes. In bacteria, the major stress proteins, hsp70 and hsp60, occur at moderate levels in cells that have not been stressed but accumulate to very high levels in stressed cells. For example, hsp70 and hsp60 normally account for 1-3% of total E. coli protein, but can accumulate to about 25% under stressful conditions. Eukaryotic hsp70 and hsp60 proteins do not accumulate to these extreme levels. Their levels range from undetectable to moderately abundant, depending on the organism and cell type.

[0017] The present invention is based on the observation that stress proteins are among the major antigens available for presentation to T lymphocytes and may be common immune targets in a broad spectrum of infectious diseases. Immune responses to stress proteins are involved in immune surveillance by the body and a variety of different T cell types has been shown to recognize highly conserved stress protein determinants. Several observations, described below, suggest a model of immune surveillance in which self-reactive T cells provide a first line of defense against infection or other invasion by pathogens, which include, but are not limited to, viruses, microorganisms, other organisms, substances such as toxins and toxoids, and agents which cause cell transformation, by recognizing and helping to eliminate stressed autologous cells, as well as cells infected with intracellular pathogens. Without wishing to be bound by this model, it is presented as one means by which it is possible to explain why prokaryotic and eukaryotic cells respond to a variety of potentially damaging stimuli, such as elevated temperature, by increasing the synthesis of a family of proteins, referred to as stress proteins, which are among the most highly conserved and abundant proteins found in nature.

[0018] Investigation of antigens involved in the immune response to the tuberculosis and leprosy bacilli (M. tuberculosis and M. leprae) initially led to the observation that a variety of stress proteins are among the major targets of the immune response, as is described at greater length below.

[0019] Further assessment has demonstrated that stress proteins may be common immune targets in a broad spec-

trum of infectious diseases. Sequence analysis has revealed 70-kDa heat shock protein homologues among major antigens of the protozoan parasites Plasmodium falciparum (Bianco, A.E. et al., Proc. Natl. Acad. Sci., USA, 83: 8713-8717 (1986)) and Schistosoma mansoni (Hedstrom, R. et al., J. Exp. Med., 165:1430-1435 (1987)) and the malarial parasite Brugia malayi (Selkirk, M.E. et al., J. Cell Biochem., 12D:290 (1988)). Similarly, homologues of GroEL have been found among antigens involved in the immune response to Salmonella typhimurium and Coxiella (Vodkin, M.H. and J.C. Williams, J. Bacteriol, 170:1227 (1988)), as well as Bordetella pertussis (Del Giudice, G., et al., J. of Imm., 150: 2025-2032 (1993)). The presence of stress proteins among major immune targets in a variety of human pathogens is support for the idea that the stress response may be a general component of infection and that stress proteins should be considered among candidates for subunit vaccines. All organisms respond to heat by inducing synthesis of heat shock proteins (hsp), which are a group of proteins. This response is the most highly conserved genetic system known and has been shown to occur in every organism, including microorganisms, plants and animals, investigated to date. Many of the characteristics of the response are common to all organisms and the hsp are among the most highly conserved proteins known. For example, hsp90 family and hsp70 family proteins are present in widely diverse organisms. The proteins in each family-- even in such diverse organisms--show approximately 50% identity at the amino acid level and at the nonidentical residues, exhibit many similarities. Several of the proteins induced by heat are also induced by a variety of other stresses. The hsps or a closely related/similar protein are present in all organisms at normal temperatures and have been shown to have key functions in normal cell metabolism. Lindquist, S. and E.A. Craig, Ann. Rev. Genet., 22:631-677 (1988). Because the stress response is common to prokaryotes and eukaryotes and stress proteins are among the most highly conserved in sequence, it is reasonable to expect that an antigen from one pathogen could immunize against another pathogen. Exposure to foreign stress proteins early in life might, in fact, induce a degree a immunity to a variety of infectious agents. If so, this could provide an explanation for the observation that, for many pathogens, only a fraction of infected individuals actually acquire clinical disease.

[0020] The following is a description of the relationship which has been observed between stress proteins and the immune response to mycobacterial infection; of the observation and supporting information that stress proteins are immune targets in many infections by pathogens; of the role of stress proteins as immune targets in transformed cells; of recognition of the fact that the immune response to conserved stress protein determinants may play an important role in autoimmune pathology in rheumatoid arthritis, as well as in adjuvant arthritis; and of the role of stress proteins in immune surveillance, as well as a model proposed for immune surveillance in which self-reactive T cells provide a first line of defense against infection and cell transformation.

Mycobacterial Stress Proteins are Targets of the Immune Response

[0021] An intriguing relationship between stress proteins and the immune response to mycobacterial infection has been observed. A more detailed examination of stress protein determinants and immune response mechanisms is essential to understanding the relationship among stress proteins, infection, and immunity.

[0022] In view of the involvement of proteins of M. tuberculosis and M. leprae in humoral and cell-mediated immune responses and to establish the functions of these proteins in the mycobacterial cell, the DNA encoding several of the M. tuberculosis and M. leprae antigens have been sequenced. The results, discussed in Example 1, demonstrate that many of these mycobacterial protein antigens exhibit striking sequence similarity to known stress-induced proteins. Three of the M. leprae and two of the M. tuberculosis protein antigens studied have been shown to exhibit striking sequence similarity to known stress proteins. For reasons discussed in Example 1, it is concluded that two of the M. leprae and two of the M. tuberculosis antigens are homologues of the E. coli DnaK and GroEL proteins.

[0023] In mice, immunization with mycobacterial lysates elicits antibody responses to at least six M. tuberculosis protein antigens and a similar number of M. leprae protein antigens. Monoclonal antibodies specific for these proteins have been used to isolate clones from λgt11 DNA expression libraries of M. tuberculosis and M. leprae. The sequence of the DNA clones revealed that mycobacterial hsp70 (alias 70 kDa antigen) and hsp60 (alias 65 kDa antigen, GroEL) were the major targets of the murine antibody response to both M. tuberculosis and M. leprae. Two additional hsp, an 18 kDa member of the small hsp family and a 12 kDa homologue of groES, were found among the M. leprae and M. tuberculosis antigens. Young, D.B., et al., Proc. Natl. Acad. Sci., USA, 85:4267-4270 (1988); Shinnick, T.M., et al., Nuc. Acids Res., 17:1254 (1989).

[0024] The mycobacterial stress proteins are among the immunodominant targets of both murine antibody and T cell responses. In one study which summarized results obtained from 10 laboratories, a collection of 24 murine monoclonal antibodies recognized 6 M. leprae proteins; 7 of these antibodies are directed against 6 different determinants in the M. leprae hsp60. Engers, H.D., et al., Infect. Immun., 48:603-605 (1985); Mehra, V., et al., Proc. Natl. Acad. Sci., USA, 83:7013-7017 (1986). In a similar study, 3 of 33 monoclonal antibodies raised against M. tuberculosis recognized the M. tuberculosis hsp60 protein. Engers, H.D., et al., Infect. Immun., 51:718-720 (1986). Finally, limiting dilution analysis indicates that 20% of the mycobacterial-reactive CD4+ T lymphocytes in mice immunized with M. tuberculosis recognize this antigen. Kaufmann, S.H., et al., Eur. J. Immunol., 17:351-357 (1987).

[0025] Although a rigorous quantitative analysis of the human immune response to mycobacterial stress proteins has not yet been reported, mycobacterial stress proteins are recognized by human antibodies and T lymphocytes and the evidence suggests that these proteins are among the major targets of the human cell mediated immune response. Emmrich. F., et al., J. Exp. Med., 163:1024-1029 (1985); Mustafa, A.S., et al., Nature (London). 319:63-66 (1986); Oftung, F., et al., J. Immunol., 138:927-931 (1987); Lamb, J.R., et al., EMBO J., 6:1245-1249 (1987). T lymphocytes from patients with mycobacterial infection or from volunteers immunized with mycobacteria have been cloned and tested for their ability to recognize the mycobacterial stress proteins. In each of these studies, some fraction of the human T cell clones were shown to recognize one or more of the mycobacterial stress proteins.

Stress Proteins are Immune Targets in Infections by Pathogens

[0026] The observation that stress proteins are important targets of the immune response to mycobacterial infection and the knowledge that the major stress proteins are conserved and abundant in other organisms suggested that stress proteins are likely to be immune targets in many infections by pathogens. Indeed, that is now clearly the case. Antigens from a wide variety of infectious agents have been identified as members of stress protein families. The major stress protein antigen recognized by antibodies in bacterial infections is hsp60. "Common antigen", an immunodominant protein antigen long known to be shared by most bacterial species, turns out to be hsp60. Shinnick, T.M., et al., Infect. Immun., 56:446 (1988); Thole, J.E.R., et al., Microbial Pathogenesis, 4:71-83 (1988). Stress proteins have also been identified as immune targets in most major human parasite infections. Bianco, A.E., et al., Proc. Natl. Acad. Sci. USA, 83:8713 (1986); Nene, V., et al., Mol. Biochem. Parasitol., 21:179 (1986); Ardeshir, F., et al., EMBO J., 6:493 (1987); Hedstrom, R., et al., J. Exp. Med., 165:1430 (1987); Selkirk, M.E., et al., J. Cell Biochem., 12D:290 (1988), Engman, D.M., et al., J. Cell Biochem., 12D: Supplement, 290 (1988); Smith, D.F., et al., J. Cell Biochem., 12D:296 (1988). Antibodies to hsp70 have been identified in the sera of patients suffering from malaria, trypanosomiasis, leishmaniasis, schistosomiasis and filariasis. Hsp90 is also a target of antibodies in trypanosomiasis and a member of the small hsp family is recognized in some patients with schistosomiasis.

[0027] Proteins homologous to stress proteins have also been identified in viruses. Recently, a protein encoded by the RNA genome of the Beet Yellows Closterovirus, a plant virus, has been shown to be homologous to hsp70. Agranovsky, A.A., et al., J. Mol. Biol., 217: 603-610 (1991). In addition, stress protein induction occurs in eukaryotic cells following infection by diverse viruses in vitro. Collins, P.L., and Hightower, L.E., J. Virol., 44:703-707 (1982); Nevins, J.R., Cell, 29:913-939 (1982); Garry, R.F. et al., Virology, 129:391-332 (1988); Khandjian, E.W. and Turler, H., Mol. Cell Biol., 3:1-8 (1983); LaThangue, N.B., et al., EMBO J., 3:267-277 (1984); Jindal, S. and Young, R., J. Viral, 66:5357-5362 (1992). CTL that recognize these neo-antigens could limit the spread of virus by killing infected cells, possibly before substantial amounts of mature virus are assembled, and by secreting the lymphokine γ-interferon. Pestka, S., in: Methods Enzymol., Interferons, Part A., Vol. 79 Academic Press, New York, pp. 667 (1981). Evidence consistent with this idea is emerging. Koga et al., (1989) have shown that infection of primary murine macrophages with CMV rendered them susceptible as targets for MHC-I restricted CD8+ CTL specific for linear epitopes of M. tuberculosis hsp60. Koga, T., et al. (1989). Although the epitope recognized by these CTL on infected macrophages was not defined, it is tempting to speculate that a cross-reactivity with self hsp60 epitopes is being observed. Indeed, the same groups showed that a homologous hsp60 is constitutively present in macrophages and is upregulated by γ-interferon stimulation.

Stress Proteins as Immune Targets in Transformed Cells

[0028] Stress proteins appear to be produced at high levels in at least some transformed cells. Bensaude, O. and Morange, M., EMBO J., 2: 173-177 (1983). An 86 kDA murine tumor antigen has been found to be homologous to representatives of the hsp90 family in yeast and Drosophila. Ullrich, S.J., Proc. Natl. Acad. Sci., USA, 83: 3121-3125 (1986). Immunization of mice with the purified protein led to inhibition of tumor growth in 95% of experimental animals that had been seeded with cultured tumor cells. All of the protected mice had high titers of anti-hsp90 serum antibody which was able to precipitate murine hsp90 from lysates of heat shocked mouse embryo cells. Again, a role for autoreactive lymphocytes is implied, since T cells capable of recognizing autologous cells stressed by transformation could help eliminate nascent tumor cells.

Stress Proteins and Autoimmune Processes

[0029] Rheumatoid arthritis is characterized by a chronic proliferative and inflammatory reaction in synovial membranes which is thought to involve autoimmune processes. Rat adjuvant arthritis resembles human rheumatoid arthritis in many respects, and has been used as an experimental animal model for human disease. Pearson, C.M., Arthritis Rheum., 7:80-86 (1964). Adjuvant arthritis can be induced in rats with a single intradermal injection of killed M. tuber-

culosis in complete Freund's adjuvant. An autoimmune process involving T lymphocytes appears to be responsible for the generation of the disease. Holoshitz, J., et al., Science, 219:56-58 (1983). T cell lines isolated from the draining lymph nodes of arthritic rats and propagated in vitro by stimulation with M. tuberculosis-pulsed syngeneic antigen presenting cells can cause a transient form of the disease when transferred to irradiated rats. Since care was taken in these experiments to exclude the transfer of contaminating M. tuberculosis, this result strongly suggests that the clinical effects of the disease are a consequence of an autoimmune reaction in which the autoantigen is shared with M. tuberculosis.

[0030] The rat and M. tuberculosis antigens recognized by the arthritogenic T cells have been sought for a number of years. A number of different proteins present in synovial membranes have been proposed to be the cross-reactive rat antigen, but were later discounted as procedures for the purification of these proteins improved. van Eden, W., et al., Proc. Natl. Acad. Sci., USA, 82:5117-5120 (1985); Holoshitz, J., et al., Science, 219:56-58 (1983). The M. tuberculosis antigen recognized by the arthritogenic T cells was recently shown to be a 65 kDa protein (van Eden, W., et al., Nature, 331:171 (1988), which has now been shown to be hsp60 (see the Example 1). Using a combination of truncated recombinant 65 kDa proteins and peptides, a nine amino acid epitope of hsp60 has been identified as the minimum stimulatory sequence for arthritogenic T cell clones in proliferation assays. Now that it is clear that some arthritogenic T cells recognize the mycobacterial hsp60, it is quite possible that the rat autoantigen is also hsp60.

[0031] The results obtained in the adjuvant arthritis model led investigators to determine whether T lymphocytes from human rheumatoid arthritis patients also recognize mycobacterial antigens. These investigators have found not only that patients with rheumatoid arthritis have T cells that recognize M. tuberculosis antigens, but that these T cells have diverse phenotypes. Substantial proliferative responses to mycobacterial extracts are observed with uncloned T cells (predominantly $CD4^+$) from both synovial infiltrates and peripheral blood, although responses are generally greater in synovial infiltrates. Abrahamson, T.G., et al., Scand. J. Immunol., 7:81-90 (1978); Holoshitz, J., et al., Lancet ii, 305-306 (1986). Holoshitz et al. found that 4 of 5 T cell clones isolated from human rheumatoid synovia which respond to M. tuberculosis antigens were $CD4^-$ $CD8^-$ cells with γ/δ T cell receptors. Holoshitz, J., et al., Nature, 339:226-229 (1989). This observation is interesting because γ/δ T cells have yet to be assigned a role in immunity. One of the γ/δ clones was tested for its ability to respond to purified mycobacterial hsp60 and was found to be positive in proliferation assays. Due to the conserved nature of stress proteins, these T cells have the potential for autoreactivity. Lamb and coworkers have shown that polyclonal T cells from synovial infiltrates recognize both mycobacterial hsp60 and hsp70. Lamb, J.R., et al., Intl. Immunol., in press (1989). The population of T cells that recognize the mycobacterial stress proteins were shown to respond to E. coli hsp60 and hsp70 and, most interestingly, human hsp70 purified from heat shocked macrophages. Thus, immune responses to conserved stress protein determinants, perhaps initiated by bacterial infection (not necessarily by mycobacteria), may play an important role in autoimmune pathology in rheumatoid arthritis, as well as in adjuvant arthritis.

Stress Proteins and Immune Surveillance

Stress Proteins and Immune Surveillance

[0032] A variety of different T cell types has now been shown to recognize highly conserved stress protein determinants. The ability of cells to respond to stress by increasing the levels of the highly conserved stress proteins; the presence of T cells of diverse phenotypes in healthy individuals that are capable of recognizing self stress protein determinants; and observations that stress responses are induced by pathogenic infections and by cell transformation, all suggest a model of immune surveillance in which self-reactive T cells provide a first line of defense against infection and transformation by recognizing and helping to eliminate stressed autologous cells, as well as cells infected with intracellular pathogens. The pool of lymphocytes that recognize conserved stress protein determinants might be induced during establishment of natural microbial flora on the skin and in the gut, and maintained by frequent stimulation by pathogens, such as bacteria and viruses, as well as other stressful stimuli encountered during a normal lifetime. This model is attractive because it provides a way in which the immune system could exploit the existence of conserved epitopes in stress proteins to respond immediately to antigenically diverse pathogens and cellular changes, producing an initial defense that need not await the development of immunity to novel antigens.

[0033] The lymphocytes which recognize conserved stress protein determinants must be capable of discriminating between normal and stressed cells. Since many stress proteins are constitutively expressed in normal cells, although at lower levels than in stressed cells, the potential for autoreactivity is ever-present. Normal cells may escape destruction by expressing only substimulatory levels of stress protein determinants on their surfaces. In addition, stress proteins may only be processed and presented during stress, and it may be relevant that many stress proteins have altered intracellular locations during stress. Finally, immune regulatory networks may prevent activation of autoreactive T cells under normal conditions. The regulatory constraints required by this system might occasionally break down, perhaps during stress caused by bacterial or viral infections, leading to autoimmune disease. Rheumatoid arthritis may be such

a disease.

Modulation of Immune Response

[0034] The precise relationship between stress proteins and the host immune response to infection is as yet undefined. When cells are subjected to a variety of stresses, they respond by selectively increasing the synthesis of a limited set of stress proteins. Some stress proteins, including the products of DnaK and GroEL, are major constituents of the cell under normal growth conditions and are induced to even higher levels during stress. Lindquist, S., Annu. Rev, Biochem. 55: 1151-1191 (1986); Neidhardt, F.C. and R.A. VanBogelen, In Escherichia coli and Salmonella Typhimurium, Cellular and Molecular Biology, (eds. Neidhardt, F.C., Ingraham, J.L. Low, K.B. Magasanik, B. Schaechter, M. and Umbarger, H.E.) Am. Soc. Microbiol., Washington, D.C., pp. 1134-1345 (1957). It has now been demonstrated that stress-related proteins are targets of the immune response. Young, D. et al., Proc. Natl. Acad. Sci. USA, 85: 4267-4270 (1988). It is reasonable to expect that immunodominant antigens would be found among such abundant proteins, as has now been shown to be the case.

[0035] It is possible to modulate the immune response in an individual, such as a human, other mammal or other vertebrate, by altering the individual's response to stress proteins. In particular, it is possible to enhance or induce an individual's response to a pathogen (e.g., bacteria, virus, parasites, or other organism or agent, such as toxins, toxoids) or to cancer cells or enhance or induce an upregulation of an individual's immune status (such as in an immune compromised individual or HIV-infected individual); and to decrease an individual's autoimmune response, such as occurs in some forms of arthritis. In addition, administration of a stress protein using the method of the present invention provides protection against subsequent infection by a pathogen. As demonstrated herein, stress proteins contain regions of highly conserved amino acid sequences and have been shown to be major immunodominant antigens in bacterial and other infections. Therefore, it is reasonable to expect stress proteins can be used to elicit strong immune responses against a variety of pathogens. The stress protein administered to induce or enhance an immune response to pathogens can be the stress protein of the pathogen against which an immune response is desired or other stress protein, a portion of that protein of sufficient size to stimulate the desired immune response or a protein or amino acid sequence which is the functional equivalent of the stress protein in that it is sufficiently homologous in amino acid sequence to that of the stress protein to be capable of eliciting the desired response (an immune response substantially similar to that which occurs in response to the stress protein) in the individual to whom it is administered. The term "sufficiently homologous in amino acid sequence to that of the stress protein" means that the amino acid sequence of the protein or polypeptide will generally show at least 40% identity with the stress protein amino acid sequence; in some cases, the amino acid sequence of a functional equivalent exhibits approximately 50% identity with the amino acid sequence of the stress protein.

[0036] Any stress-induced proteins or their functional equivalents can be used in the fusion proteins of the invention to enhance or induce an immune response in an individual (e.g. a human, other mammal or vertebrate), against an infection by a pathogen, for immmotherapy against cancer cells, for generally upregulating an individual's immune status and for use in inducing immune tolerance in an individual or animal.

[0037] The fusion proteins of the present invention can be administered in a variety of ways to modulate the immune response of an individual (e.g., a human, other mammal or other vertebrate). The fusion protein can be administered as a vaccine which is comprised of the fusion protein comprising stress protein or a portion of the stress protein which is of sufficient size to stimulate the desired immune response. The vaccine can be a "specific vaccine" which contains a specific stress protein of a particular pathogen against which an immune response is desired, such as a bacterial stress protein. In this case, since the pathogen's stress proteins are distinguishable from those of the host, it is possible to induce an immunoprophylactic response specific to the pathogen's stress proteins. Blander, S.J., et al., J. Clin. Invest., 91:717-723 (1993). This can be carried out by administering a vaccine which includes all or a portion (e.g., sufficient amino acid sequence to have the desired stimulatory effect on immune response) of the pathogen's stress protein or of another protein having an amino acid sequence sufficiently similar to that of the stress protein sequence to stimulate the immune response to the pathogen's stress protein. Alternatively, in the case of a pathogen which does not contain stress proteins, (e.g. some viruses) or in the condition of neoplasia, fusion proteins comprising stress proteins or highly conserved stress protein determinants, such as those shown to be recognized by a variety of T cells, can be administered as a type of "general" vaccine to achieve an upregulation of the immune response. Administration of such a vaccine will enhance the existing immune surveillance system. For instance, a vaccine which includes a bacterial, or other stress protein can be administered to enhance the immune system which will result in an immune response against a pathogen which does not contain stress proteins. Alternatively, this type of "general" vaccine can be used to enhance an individual's immune response against cancer or to generally upregulate an individual's immune status, such as in an immune compromised individual (e.g., an individual undergoing chemotherapy or an HIV-infected individual). In either case (specific or general vaccine), the immune response to the stress protein sequence will be increased and effects of the pathogen, disease condition or immune impairment will be reduced (decreased, prevented

or eliminated).

[0038] Stress proteins can be used to enhance immune surveillance by applying local heat or any other substances or changes in condition which induce the stress response in the individual being treated. (This can also be employed in conjunction with the specific vaccine, described previously, administered to enhance an immune response to a stress protein-containing pathogen or in conjunction with the general vaccine, described above, administered to enhance the immune response against a pathogen which does not contain its own stress proteins, cancer, or to upregulate the immune status of an individual). For example, it is known that increased levels of stress proteins are produced in many types of cancer cells. Therefore, enhancement of the immune surveillance system can be used to facilitate destruction and/or to prevent progression or establishment of cancer cells.

[0039] The present invention also finds application in the modification or modulation of an individual's response to his or her own cells (e.g., as in autoimmune diseases). There are at least two ways in which the present invention can be used immunotherapeutically. First, stress proteins, such as heat shock proteins (e.g., hsp 70 and hsp60), are known to be involved in autoimmune disease. It is, thus, possible to turn down an individual's immune response, resulting in the individual becoming more tolerant of the protein. Second, because it is known that under some circumstances, one component of the immune response in certain autoimmune diseases can be to stress proteins, it is possible to selectively inhibit or interfere with the ability of immune cells which normally interact with such proteins to do so. This can be done, for example, by administering monoclonal antibodies that bind to specific T cell receptors and delete or disable such cells. Alternatively, rather than knocking out immune cells, the stress response in cells can be turned down by administering a drug capable of reducing a cell's ability to undergo the stress response. For example, a drug targeted to or specific for heat shock transcription factor, which is needed to stimulate heat shock genes, can be administered. The transcription factor is rendered nonfunctional or subfunctional and, as a result, cells' ability to undergo the stress response is also lessened.

[0040] The stress protein may be administered as a vaccine which is comprised of two moieties: a stress protein and another substance (referred to as an antigen, e.g. protein or peptide) against which an immune response is desired. The two moieties are joined to form a single unit by recombinant techniques.(Example. 2.). The result is a recombinant fusion protein which includes the stress protein and the antigen in a single molecule. This makes it possible to produce and purifly a single recombinant molecule in the vaccine production process. The stress protein can be seen to act as an adjuvant-free carrier, and it stimulates strong humoral and T cell responses to the substance to which the stress protein is fused.

[0041] As demonstrated in Example 3, the HIV p24 gag gene was subcloned into the stress protein fusion vector pKS70 (Figure 6), containing the T7 RNA polymerase promoter, a polylinker and the *mycobacterial tuberculosis* hsp70 gene. The resulting vector pKS72 (Figure 6) was used to produce the p24-hsp70 fusion protein in *E. coli.* Adjuvant-free, purified p24-hsp70 fusion protein was injected into Balb/c mice and as shown in Figure 7, the anti-p24 antibody titer was 2.7 orders of magnitude higher in mice injected with the p24-hsp70 fusion protein than in mice injected with p24 alone or hsp70 alone. Mice injected with p24 and the adjuvant, alum, also produced an antibody response to p24. Finally, a demonstrable T cell response was seen in mice injected with the p24-hsp70 fusion protein and in mice injected with p24 alone.

[0042] The stress protein, stress protein portion, stress protein functional equivalent and the substance to which the stress protein is fused can be produced or obtained using known techniques. For example, the stress protein or stress protein portion can be obtained (isolated) from a source in which it occurs in nature, can be produced by cloning and expressing a gene encoding the desired stress protein or stress protein portion or can be synthesized chemically or mechanically.

[0043] An effective dosage of the fusion proteins of the present invention, to elicit specific cellular and humoral immunity to fusion proteins, is in the range of 0.1 to 1000 ug per injection, depending on the individual to whom the fusion protein is being administered. Lussow, A.R., et al, Eur. J. Immun., 21:2297-2302 (1991). Barrios, C. et al., Eur. J. Immun., 22:1365-1372 (1992). The appropriate dosage of the fusion protein for each individual will be determined by taking into consideration, for example, the particular fusion protein being administered, the type of individual to whom the fusion protein is being administered, the age and size of the individual, the condition being treated or prevented and the severity of the condition. Those skilled in the art will be able to determine using no more than routine experimentation, the appropriate dosage to administer to an individual.

[0044] Various delivery systems can be used to administer an effective dose of the vaccine of the present invention. Methods of introduction include, for example, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural and oral routes. Any other convenient route of administration can be used (infusion of a bolus injection, infusion of multiple injections over time, absorption through epithelial or mucocutaneous linings such as, oral mucosa, rectal and intestinal mucosa) or a series of injections over time.

[0045] The present invention is further illustrated by the following exemplification, which is not intended to be limiting in any way.

EXEMPLIFICATION

EXAMPLE 1 Isolation and Characterization of Mycobacterial Stress Protein Antigens

[0046] Recombinant DNA Clones. The isolation and characterization of M. tuberculosis and M. leprae λgtII genomic DNA clones with murine monoclonal antibodies have been described. Husson, R.N. and Young, R.A., Proc. Natl. Acad. Sci., USA 84: 1679-1683 (1987); Young, R.A., et al., Nature (London) 316: 450-452 (1985). DNA was isolated from these clones and was manipulated by standard procedures. Davis, R.W., Advanced Bacterial Genetics: A Manual for Genetic Engineering (Cold Spring Harbor Lab., Cold Spring Harbor, NY), (1980).

[0047] DNA Sequence Analysis. DNA was subcloned into vector M13mp18 or M13mp19 (New England Biolabs), as suggested by the supplier. Dideoxynucleotide chain-termination reactions and gel electrophoresis of the sequenced produced were as described. Davis, R.W., Advanced Bacterial Genetics: A Manual for Genetic Engineering (Cold spring Harbor Lab., Cold Spring Harbor, NY), (1980). DNA sequences were determined for both strands of DNA. Computer analysis of sequences with UWGCG programs was as described by Devereux, J., et al., Nucleic Acids Res., 12: 387-395 (1984).

[0048] Immunoblot Analysis. Escherichia coli strain TG1 was transformed with the following plasmids by standard procedures (Maniatis, T., et al., Molecular Cloning, A Laboratory Manual (Cold Spring Harbor Lab., Cold Spring Harbor, NY) (1982), with selection for ampicillin resistance: pND5, a derivative of pBR325 containing the E. coli GroEL genes (Jenkins, A.J., et al., Mol. Gen. Genet., 202: 446-454 (1986); pUC8 (Vic, J., Gene, 19: 259-268 (1982); pUC8 with insert DNA for λgt11 clone Y3178 (M. leprae 65-kDa antigen, Young, R.A., et al., Nature, (London) 316: 450-452 (1985)) ligated in the EcoRI site.

[0049] Overnight cultures of E. coli strains in Luria-Bertani (LB) medium were centrifuged and resuspended in isotonic phosphate-buffered saline at a cell density corresponding to an absorbance of 2 at 600 nm. An equal volume of sample buffer containing 2% (wt/vol) $NaDodSo_4$ was added, and, after heating on a boiling water bath for 2 min, samples were electrophoresed on 12% (wt/vol) polyacrylamide gels in the presence of $NaDodSO_4$. Blots were prepared by electrophoretic transfer of the proteins to a nitrocellulose membrane, and binding of monoclonal antibodies was assayed with a peroxidase-conjugated secondary antibody as described. Young, D.B., et al., Infect. Immun., 55: 1421-1425 (1987).

[0050] Six M. tuberculosis and six M. leprae proteins have been implicated in the immune response to the mycobacterial pathogens (Table 1). To obtain clues to the normal cellular function of several of these mycobacterial antigens, DNA clones encoding these proteins, isolated by using monoclonal antibodies to probe lambda gtII libraries (Husson, R.N. and Young, R.A., Proc. Natl. Acad. Sci., USA, 84: 1679-1683 (1987); Young, R.A., et al., Nature, (London) 316: 450-452 (1985)) were subjected to sequence analysis. The sequences elucidated have been submitted to the GenBank sequence database.

[0051] The Mycobacterial 71-k Da Antigen. The 71-k Da antigen of M. tuberculosis is recognized by human T cells during infection (Table 1).

TABLE 1

MYCOBACTERIAL PROTEIN ANTIGENS			
Protein, kDA	Recognized by Human T Cells	Subjected to sequence analysis	Homology with known proteins
M. tuberculosis			
71	+	+	DnaK
65*	+	+	GroEL
38	+	-	-
19	+	+	None
14	+	-	-
12	ND	-	-
M. leprae			
70	ND	-	DnaK
65	+	+	GroEL
36	+	-	-
28	+	-	-
18	+	+	Plant Hsp
12	ND	-	-

Mycobacterial protein antigens, their recognition by human T cells, and homology of the deduced mycobacterial protein sequences to known proteins are summarized. ND, not determined; +, yes; -, no

* Includes data derived from study of the 65-kDA antigens of M. bovis BCG (Bacillus Calmette-Gurein), which is identical to the M. tuberculosis 65-kDA antigen.

+ A.S. Mustafa, J.R. Lamb, D. Young and R.A. Young, unpublished data.

[0052] The insert DNA of lambdagtII clone Y3271 (Husson, R.N., et al., Proc. Natl. Acad. Sci, USA, 84: 1679-1683 (1987), was sequenced to obtain amino acid sequence information for the 71-kDa antigen of M. tuberculosis. This clone produces a beta-galactosidase fusion protein containing the carboxyl-terminal one-third of the 71-kDa antigen exhibiting 40% amino acid sequence identity with the comparable segment of the dnaK gene product from E. coli (Bardwell, J.C., et al., Proc. Natl. Sci., USA, 81: 848-852 (1984)), (Fig. 1). Figure 1A shows the extent of sequence similarity between portions of the mycobacterial and the E. coli 70-k Da polypeptides. Sequences transcriptionally downstream from the mycobacterial 71-k Da gene predict a 356-amino acid protein homologous to the E. coli dnaJ gene product (unpublished data), indicating that the E. coli dnaK-dnaJ operon structure is conserved in M. tuberculosis and consistent with the conclusion that the mycobacterial 71-kDa antigen is a homologue of the E. coli dnaK gene product. The product of the dnaK gene is a member of the 70-kDa heat shock protein family that is highly conserved among prokaryotes and eukaryotes (Bardwell, J.C., et al., Proc. Natl. Acad. Sci., USA, 81: 848-852 (1984); Lindquist, S., Annu. Rev. Biochem., 55: 1151-1191 (1986).

[0053] The M. leprae 70-k Da antigen cross-reacts with monoclonal antibodies directed to the M. tuberculosis 70-kDa antigen. M. tuberculosis and M. leprae are both members of the 70-k Da heat shock protein family of stress proteins.

[0054] The mycobacterial 65-kDa antigen. The 65-kDa antigens of M. tuberculosis and M. leprae are involved in the human T-cell response to mycobacterial infection (Table 1). Genes encoding these proteins have been isolated (Husson, R.N., and Young, R.A., Proc. Natl. Acad. Sci., USA, 84: 1679-1683 (1987); Young, R.A., et al., Nature, (London) 316: 450-452 (1985)) and sequenced (Shinnick, T.M., J. Bacteriol., 169: 1080-1088 (1987); Mehram, V., et al., Proc. Natl. Acad. Sci., USA 83: 7013-7017 (1986)), revealing that the amino acid sequences of the 65-kDa antigens of M. tuberculosis (SEQ ID NO: 4) and M. leprae (SEQ ID NO: 3) are 95% identical. These proteins sequences exhibited no significant sequence similarity to proteins in the GenBank database.

[0055] Identification of these proteins was based on the observation that some monoclonal antibodies directed against the mycobacterial 65-kDa antigens cross-react with an E. coli protein of 60kDa. E. coli cells transformed with the plasmid pND5 (Sanger, F., et al., Proc. Natl. Acad. Sci., USA 74: 5463-5467 (1977), which contains the E. coli gro E genes, had been shown to accumulate large amounts of the 60-kDa protein. A comparison of the mycobacterial

65-kDa protein sequences with those determined for E. coli groEl (C. Woolford, K. Tilly, C. Georgopoulous, and R.H., unpublished data) revealed the extent of the sequence similarity as shown in Figure 1B.

[0056] The 60-kDa Gro EL protein is a major stress protein in E. coli. Lindquist, S., Annual. Rev. Biochem., 55: 1151-1191 (1986); Nature, 333: 330-334 (1988). There is some evidence that the mycobacterial 65-kDa proteins accumulate in response to stress: Mycobacterium bovis BCG (bacillus Calmette-Guerin) cultures grown in zincdeficient medium are substantially enriched in this protein (De Bruyn, J., et al., Infect. Immun. 55: 245-252 (1987)). This infers that the 65-kDa proteins of M. tuberculosis and M. leprae are homologues of the E. coli Gro EL protein.

[0057] Other Mycobacterial Antigens. T lymphocytes that respond to the M. tuberculosis 19-kDa antigen and the M. leprae 18-kDa antigen have been observed in humans with tuberculosis and leprosy, respectively (Table 1). DNA encoding these antigens was sequenced from the λgtll clones Y3148 (Husson, R.N. and Young, R.A., Proc. Natl. Acad. Sci., USA 84: 1679-1683 (1987); and Y3179 (Young, R.A., et al., Nature, (London) 316: 450-452 (1985)), respectively. The M. tuberculosis 19-kDa protein sequence predicted from the DNA exhibited no significant sequence similarity to proteins in the GenBank database.

[0058] However, the M. leprae 18-kDa protein sequence was similar to the soybean 17-kDa protein heat shock protein, a protein representation of a major class of plant heat shock proteins (Schoffl, F. and Van Bogelen, R.A., In: Escherichia coli and Salmonella typhimurium, Cellular and Molecular Biology, Am. Soc. Microbiol., Washington, D.C. (1987).

EXAMPLE 2 Construction of Stress Protein-Fusion Vaccines for Use as Adjuvant-Free Carriers in Immunizations

[0059] Recombinant Fusion Vectors. A series of stress protein fusion vectors for use in E. coli were constructed and are shown in Figure 5. These vectors contain the T7 RNA polymerase promoter fused to the M. bovis BCG hsp70 gene or the M. bovis BCG hsp60 gene. The vectors also contain a polylinker with multiple cloning sites, permitting incorporation of a gene of interest so that the antigen encoded by that gene is expressed as a fusion protein with the stress protein. A subset of these vectors permit incorporation of the foreign gene with a coding sequence for a C-terminal 6-Histidine "tag" for ease of fusion protein purification. Thus far, recombinant clones have been generated that produce hsp70 proteins fused to HIV gag and HIV pol proteins.

[0060] Purification of stress protein fusions. Two strategies have been developed to purify the recombinant fusion proteins. The T7 system usually produces such large amounts of protein that it forms inclusion bodies, permitting purification by centrifugation. The preliminary results indicate that an hsp70-HIV gag fusion protein accounts for about 20% of total E. coli protein in the T7 system. If necessary, other fusion proteins can be purified via the 6-Histidine "tag".

EXAMPLE 3 ADJUVANT-FREE CARRIER EFFECT OF HSP70 *IN VIVO*

[0061] The stress protein fusion vector pKS70 (figure 6), containing the T7 RNA polymerase promoter, a polylinker and the *mycobacterial tuberculosis* hsp70 gene, was constructed. The HIV p24 gag gene was subcloned into pKS70 using the NdeI and BamHI sites and the resulting pKS72 vector (Figure 6) was used to produce the p24-hsp70 fusion protein in *E. coli*. The fusion protein was purified as inclusion bodies and further purified using ATP-agarose chromatography and MonoQ ion exchange chromatography.

[0062] The p24-hsp70 protein in phosphate buffered saline (PBS), in the absence of an adjuvant, was injected intraperitoneally into Balb/c mice. As controls, the p24 protein alone in PBS or the hsp70 protein alone in PBS was injected into different groups of mice. Three weeks later, the mice were boosted and finally, three weeks after the boost, the mice were bled. The anti-p24 antibody titer was then determined by ELISA. Mice injected with 25 pmoles of p24-hsp70 had antibody levels 2.7 orders of magnitude higher than mice injected with p24 alone or hsp70 alone (Figure 7). Results of experiments in which mice were injected with p24 and the adjuvant, alum, also showed that there was an antibody response to p24. In addition, mice injected with the p24-hsp70 fusion protein and mice injected with p24 alone produced a demonstrable T cell response.

Equivalents

[0063] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are encompassed in the scope of the following claims.

SEQUENCE LISTING

[0064]

(1) GENERAL INFORMATION:

(i) Applicants: Whitehead Institute for Biomedical Research and Medical Research Council

(ii) TITLE OF INVENTION: Stress Proteins and Uses Therefore

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Hamilton, Brook, Smith & Reynolds, P.C.
(B) STREET: 2 Militia Drive
(C) CITY: Lexington
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02173

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/073,381
(B) FILING DATE: 04 June 1993

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Granahan, Patricia
(B) REGISTRATION NUMBER: 32,227
(C) REFERENCE/DOCKET NUMBER: WHI88-08AFA2

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (617) 861-6240

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 575 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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            20                  25                  30

Gly Ala Asp Ala Arg Ala Leu Met Leu Gln Gly Val Asp Leu Leu Ala
        35                  40                  45

Asp Ala Val Ala Val Thr Met Gly Pro Lys Gly Arg Thr Val Ile Ile
    50                  55                  60

Glu Gln Ser Trp Gly Ser Pro Lys Val Thr Lys Asp Gly Val Thr Val
65                  70                  75                  80
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Ala Lys Ser Ile Asp Leu Lys Asp Lys Tyr Lys Asn Ile Gly Ala Lys
85 90 95

Leu Val Gln Asp Val Ala Asn Asn Thr Asn Glu Glu Ala Gly Asp Gly
100 105 110

Thr Thr Thr Ala Thr Val Leu Ala Arg Ser Ile Ala Lys Glu Gly Phe
115 120 125

Glu Lys Ile Ser Lys Gly Ala Asn Pro Val Glu Ile Arg Arg Gly Val
130 135 140

Met Leu Ala Val Asp Ala Val Ile Ala Glu Leu Lys Lys Gln Ser Lys
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Pro Val Thr Thr Pro Glu Glu Ile Ala Gln Val Ala Thr Ile Ser Ala
165 170 175

Asn Gly Asp Lys Glu Ile Gly Asn Ile Ile Ser Asp Ala Met Lys Lys
180 185 190

Val Gly Arg Lys Gly Val Ile Thr Val Lys Asp Gly Lys Thr Leu Asn
195 200 205

Asp Glu Leu Glu Ile Ile Glu Gly Met Lys Phe Asp Arg Gly Tyr Ile
210 215 220

Ser Pro Tyr Phe Ile Asn Thr Ser Lys Gly Gln Lys Cys Glu Phe Gln
225 230 235 240

Asp Ala Tyr Val Leu Leu Ser Glu Lys Lys Ile Ser Ser Ile Gln Ser
245 250 255

Ile Val Pro Ala Leu Glu Ile Ala Asn Ala His Arg Lys Pro Leu Val
260 265 270

Ile Ile Ala Glu Asp Val Asp Gly Glu Ala Leu Ser Thr Leu Val Leu
275 280 285

Asn Arg Leu Lys Val Gly Leu Gln Val Val Ala Val Lys Ala Pro Gly
290 295 300

Phe Gly Asp Asn Arg Lys Asn Gln Leu Lys Asp Met Ala Ile Ala Thr
305 310 315 320

Gly Gly Ala Val Phe Gly Glu Glu Gly Leu Thr Leu Asn Leu Glu Asp
325 330 335

Val Gln Pro His Asp Leu Gly Lys Val Gly Glu Val Ile Val Thr Lys
340 345 350

Asp Asp Ala Met Leu Leu Lys Gly Lys Gly Asp Lys Ala Gln Ile Glu
355 360 365

Lys Arg Ile Gln Glu Ile Ile Glu Gln Leu Asp Val Thr Thr Ser Glu
370 375 380

Tyr Glu Lys Glu Lys Leu Asn Glu Arg Leu Ala Lys Leu Ser Asp Gly
385 390 395 400

Val Ala Val Leu Lys Val Gly Gly Thr Ser Asp Val Glu Val Asn Glu
405 410 415

Lys Lys Asp Arg Val Thr Asp Ala Leu Asn Ala Thr Arg Ala Ala Val
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Glu Glu Gly Ile Val Leu Gly Gly Gly Cys Ala Leu Leu Arg Cys Ile
435 440 445

Pro Ala Leu Asp Ser Leu Thr Pro Ala Asn Glu Asp Gln Lys Ile Gly
450 455 460

Ile Glu Ile Ile Lys Arg Thr Leu Lys Ile Pro Ala Met Thr Ile Ala
465 470 475 480

Lys Asn Ala Gly Val Glu Gly Ser Leu Ile Val Glu Lys Ile Met Gln
485 490 495

Ser Ser Ser Glu Val Gly Tyr Asp Ala Met Ala Gly Asp Phe Val Asn
500 505 510

Met Val Glu Lys Gly Ile Ile Asp Pro Thr Lys Val Val Arg Thr Ala
515 520 525

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 575 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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            20                  25                  30

Gly Asn Asp Ala Arg Val Lys Met Leu Arg Gly Val Asn Val Leu Ala
        35                  40                  45

Asp Ala Val Lys Val Thr Leu Gly Pro Lys Gly Arg Asn Val Val Leu
    50                  55                  60

Asp Lys Ser Phe Gly Ala Pro Thr Ile Thr Lys Asp Gly Val Ser Val
65                  70                  75                  80

Ala Arg Glu Ile Glu Pro Glu Asp Lys Phe Glu Asn Met Gly Ala Gln
                85                  90                  95
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Met Val Lys Glu Val Ala Ser Lys Ala Asn Asp Ala Ala Gly Asp Gly
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Thr Thr Thr Ala Thr Val Leu Ala Gln Ala Ile Ile Thr Glu Gly Leu
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Lys Ala Val Ala Ala Gly Met Asn Pro Met Asp Leu Lys Arg Gly Ile
130 135 140

Asp Lys Ala Val Thr Ala Ala Val Glu Glu Leu Lys Ala Leu Ser Val
145 150 155 160

Pro Cys Ser Asp Ser Lys Ala Ile Ala Gln Val Gly Thr Ile Ser Ala
165 170 175

Asn Ser Asp Glu Thr Val Gly Lys Leu Ile Ala Glu Ala Met Asp Lys
180 185 190

Val Gly Lys Glu Gly Val Ile Thr Val Glu Asp Gly Thr Gly Leu Gln
195 200 205

Asp Glu Leu Asp Val Val Glu Gly Met Gln Phe Asp Arg Gly Tyr Leu
210 215 220

Ser Pro Tyr Phe Ile Asn Lys Pro Glu Thr Gly Ala Val Glu Leu Glu
225 230 235 240

Ser Pro Phe Ile Leu Leu Ala Asp Lys Lys Ile Ser Asn Ile Arg Glu
245 250 255

Met Leu Pro Val Leu Glu Ala Val Ala Lys Ala Gly Lys Pro Leu Leu
260 265 270

Ile Ile Ala Glu Asp Val Glu Gly Glu Ala Leu Ala Thr Ala Val Val
275 280 285

Asn Thr Ile Arg Gly Ile Val Lys Val Ala Ala Val Lys Ala Pro Gly
290 295 300

Phe Gly Asp Arg Arg Lys Ala Met Leu Gln Asp Ile Ala Thr Leu Thr
305 310 315 320

Gly Gly Thr Val Ile Ser Glu Glu Xaa Ile Gly Met Glu Leu Glu Lys
325 330 335

Ala Thr Leu Glu Asp Leu Gly Gln Ala Lys Arg Val Val Ile Asn Lys
340 345 350

Asp Thr Thr Thr Ile Ile Asp Gly Val Gly Glu Glu Ala Ala Ile Gln
355 360 365

Gly Arg Val Ala Gln Ile Arg Gln Gln Ile Glu Glu Ala Thr Ser Asp
370 375 380

Tyr Asp Arg Glu Lys Leu Gln Glu Arg Val Ala Lys Leu Ala Gly Gly
385 390 395 400

Val Ala Val Ile Lys Val Gly Ala Ala Thr Glu Val Glu Met Lys Glu
405 410 415

Lys Lys Ala Arg Val Glu Asp Ala Leu His Ala Thr Arg Ala Ala Val
420 425 430

Glu Glu Gly Val Val Ala Gly Gly Gly Val Ala Leu Ile Arg Val Ala
435 440 445

Ser Lys Leu Ala Asp Leu Arg Gly Gln Asn Glu Asp Gln Asn Val Val
450 455 460

Ser Ser Ser Leu Xaa Arg Ala Met Glu Ala Pro Leu Arg Gln Ile Val
465 470 475 480

Leu Asn Cys Gly Glu Glu Pro Ser Val Val Ala Asn Thr Val Lys Gly
485 490 495

Gly Asp Gly Asn Tyr Gly Tyr Asn Ala Ala Thr Glu Glu Tyr Gly Asn
500 505 510

Met Ile Asp Met Gly Ile Leu Asp Pro Thr Lys Val Thr Arg Ser Ala
515 520 525

Leu Gln Tyr Ala Ala Ser Val Ala Gly Leu Met Ile Thr Thr Glu Cys
530 535 540

Met Val Thr Asp Leu Pro Lys Asn Asp Xaa Ala Ala Asp Leu Gly Ala
545 550 555 560

Ala Gly Gly Met Gly Gly Met Gly Gly Met Gly Gly Met Met Xaa
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 573 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ala Lys Thr Ile Ala Tyr
20 25 30

Asp Glu Glu Ala Arg Arg Gly Leu Glu Arg Gly Leu Asn Ser Leu Ala
35 40 45

Asp Ala Val Lys Val Thr Leu Gly Pro Lys Gly Arg Asn Val Val Leu
50 55 60

Glu Lys Lys Trp Gly Ala Pro Thr Ile Thr Asn Asp Gly Val Ser Ile
65 70 75 80

Ala Lys Glu Ile Glu Leu Glu Asp Pro Tyr Glu Lys Ile Gly Ala Glu
85 90 95

Leu Val Lys Glu Val Ala Lys Lys Thr Asp Asp Val Ala Gly Asp Gly
100 105 110

Thr Thr Thr Ala Thr Val Leu Ala Gln Ala Leu Val Lys Glu Gly Leu
115 120 125

Arg Asn Val Ala Ala Gly Ala Asn Pro Leu Gly Leu Lys Arg Gly Ile
130 135 140

Glu Lys Ala Val Asp Lys Val Thr Glu Thr Leu Leu Lys Asp Ala Lys
145 150 155 160

Glu Val Glu Thr Lys Glu Gln Ile Ala Ala Thr Ala Ala Ile Ser Ala
165 170 175

Xaa Gly Asp Gln Ser Ile Gly Asp Leu Ile Ala Glu Ala Met Asp Lys
180 185 190

Val Gly Asn Glu Gly Val Ile Thr Val Glu Glu Ser Asn Thr Phe Gly
195 200 205

Leu Gln Leu Glu Leu Thr Glu Gly Met Arg Phe Asp Lys Gly Tyr Ile
210 215 220

Ser Gly Tyr Phe Val Thr Asp Ala Glu Arg Gln Glu Ala Val Leu Glu
225 230 235 240

Glu Pro Tyr Ile Leu Leu Val Ser Ser Lys Val Ser Thr Val Lys Asp
245 250 255

Leu Leu Pro Leu Leu Glu Lys Val Ile Gln Ala Gly Lys Ser Leu Leu
260 265 270

Ile Ile Ala Glu Asp Val Glu Gly Glu Ala Leu Ser Thr Leu Val Val
275 280 285

Asn Lys Ile Arg Gly Thr Phe Lys Ser Val Ala Val Lys Ala Pro Gly
290 295 300

Phe Gly Asp Arg Arg Lys Ala Met Leu Gln Asp Met Ala Ile Leu Thr
305 310 315 320

Gly Ala Gln Val Ile Ser Glu Glu Xaa Val Gly Leu Thr Leu Glu Asn
325 330 335

Thr Asp Leu Ser Leu Leu Gly Lys Ala Arg Lys Val Val Met Thr Lys
340 345 350

Asp Glu Thr Thr Ile Val Glu Gly Ala Gly Asp Thr Asp Ala Ile Ala
355 360 365

Gly Arg Val Ala Gln Ile Arg Thr Glu Ile Glu Asn Ser Asp Ser Asp
370 375 380

Tyr Asp Arg Glu Lys Leu Gln Glu Arg Leu Ala Lys Leu Ala Gly Gly
385 390 395 400

Val Ala Val Ile Lys Ala Gly Ala Ala Thr Glu Val Glu Leu Lys Glu
405 410 415

Arg Lys His Arg Ile Glu Asp Ala Val Arg Asn Ala Lys Ala Ala Val
420 425 430

Glu Glu Gly Ile Val Ala Gly Gly Gly Val Thr Leu Leu Gln Ala Ala
435 440 445

Pro Ala Leu Asp Lys Leu Lys Leu Thr Gly Asp Glu Ala Thr Xaa Gly
450 455 460

Ala Asn Ile Val Lys Val Ala Leu Glu Ala Pro Leu Lys Gln Ile Ala
465 470 475 480

Phe Asn Ser Gly Met Glu Pro Gly Val Val Ala Glu Lys Val Arg Asn
485 490 495

Leu Ser Val Gly His Gly Leu Asn Ala Ala Thr Gly Glu Tyr Glu Asp
500 505 510

Leu Leu Lys Ala Gly Val Ala Asp Pro Val Lys Val Thr Arg Ser Ala
515 520 525

Leu Gln Asn Ala Ala Ser Ile Ala Gly Leu Phe Thr Thr Xaa Glu Ala
530 535 540

Val Val Ala Asp Lys Pro Glu Lys Thr Ala Ala Pro Ala Ser Asp Pro
545 550 555 560

Thr Gly Gly Met Gly Gly Xaa Met Asp Xaa Xaa Xaa Phe
565 570

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 573 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ala Lys Thr Ile Ala Tyr
20 25 30

Asp Glu Glu Ala Arg Arg Gly Leu Glu Arg Gly Leu Asn Ala Leu Ala
35 40 45

Asp Ala Val Lys Val Thr Leu Gly Pro Lys Gly Arg Asn Val Val Leu
50 55 60

Glu Lys Lys Trp Gly Ala Pro Thr Ile Thr Asn Asp Gly Val Ser Ile
65 70 75 80

Ala Lys Glu Ile Glu Leu Glu Asp Pro Tyr Glu Lys Ile Gly Ala Glu
85 90 95

Leu Val Lys Glu Val Ala Lys Lys Thr Asp Asp Val Ala Gly Asp Gly
100 105 110

Thr Thr Thr Ala Thr Val Leu Ala Gln Ala Leu Arg Lys Glu Gly Leu
115 120 125

Arg Asn Val Ala Ala Gly Ala Asn Pro Leu Gly Leu Lys Arg Gly Ile
130 135 140

Glu Lys Ala Val Glu Lys Val Thr Glu Thr Leu Leu Lys Gly Ala Lys
145 150 155 160

Glu Val Glu Thr Lys Glu Gln Ile Ala Ala Thr Ala Ala Ile Ser Ala
165 170 175

Xaa Gly Asp Gln Ser Ile Gly Asp Leu Ile Ala Glu Ala Met Asp Lys
180 185 190

Val Gly Asn Glu Gly Val Ile Thr Val Glu Glu Ser Asn Thr Phe Gly
195 200 205

Leu Gln Leu Glu Leu Thr Glu Gly Met Arg Phe Asp Lys Gly Tyr Ile
210 215 220

Ser Gly Tyr Phe Val Thr Asp Pro Glu Arg Gln Glu Ala Val Leu Glu
225 230 235 240

Asp Pro Tyr Ile Leu Leu Val Ser Ser Lys Val Ser Thr Val Lys Asp
245 250 255

Leu Leu Pro Leu Leu Glu Lys Val Ile Gly Ala Gly Lys Pro Leu Leu
260 265 270

Ile Ile Ala Glu Asp Val Glu Gly Glu Ala Leu Ser Thr Leu Val Val
275 280 285

Asn Lys Ile Arg Gly Thr Phe Lys Ser Val Ala Val Lys Ala Pro Gly
290 295 300

Phe Gly Asp Arg Arg Lys Ala Met Leu Gln Asp Met Ala Ile Leu Thr
305 310 315 320

Gly Gly Gln Val Ile Ser Glu Glu Xaa Val Gly Leu Thr Leu Glu Asn
325 330 335

Ala Asp Leu Ser Leu Leu Gly Lys Ala Arg Lys Val Val Val Thr Lys
340 345 350

Asp Glu Thr Thr Ile Val Glu Gly Ala Gly Asp Thr Asp Ala Ile Ala
355 360 365

Gly Arg Val Ala Gln Ile Arg Gln Glu Ile Glu Asn Ser Asp Ser Asp
370 375 380

Tyr Asp Arg Glu Lys Leu Gln Glu Arg Leu Ala Lys Leu Ala Gly Gly
385 390 395 400

Val Ala Val Ile Lys Ala Gly Ala Ala Thr Glu Val Glu Leu Lys Glu
405 410 415

Arg Lys His Arg Ile Glu Asp Ala Val Arg Asn Ala Lys Ala Ala Val
420 425 430

Glu Glu Gly Ile Val Ala Gly Gly Gly Val Thr Leu Leu Gln Ala Ala
435 440 445

Pro Thr Leu Asp Glu Leu Lys Xaa Leu Glu Gly Asp Glu Ala Thr Gly
450 455 460

Ala Asn Ile Val Lys Val Ala Leu Glu Ala Pro Leu Lys Gln Ile Ala
465 470 475 480

Phe Asn Ser Gly Leu Glu Pro Gly Val Val Ala Glu Lys Val Arg Asn
485 490 495

Leu Pro Ala Gly His Gly Leu Asn Ala Gln Thr Gly Val Tyr Glu Asp
500 505 510

```
Leu Leu Ala Ala Gly Val Ala Asp Pro Val Lys Val Thr Arg Ser Ala
        515                 520                 525

Leu Gln Asn Ala Ala Ser Ile Ala Gly Leu Phe Leu Thr Thr Glu Ala
    530                 535                 540

Val Val Ala Asp Lys Pro Glu Lys Glu Lys Ala Ser Val Pro Gly Xaa
545                 550                 555                 560

Xaa Xaa Xaa Xaa Gly Gly Asp Met Gly Gly Met Asp Phe
                565                 570
```

Claims

1. A recombinant fusion protein comprising (a) a heat shock protein (hsp), or (b) a protein being at least 40% identical to said hsp, or (c) a portion of said hsp or protein, which hsp, protein or portion is capable of stimulating humoral and/or T cell responses and (d) an antigen, for use in immune therapy or prophylaxis.

2. The recombinant fusion protein of claim 1, wherein the protein is approximately 50% identical to the hsp.

3. A process for producing a recombinant fusion protein for use in immune therapy or prophylaxis, the process comprising the step of joining an hsp, protein or portion, as defined in any of claims 1 or 2, to an antigen by recombinant means.

4. Use of a recombinant fusion protein as defined in any one of claims 1 or 2 producible by the process of claim 3 for the manufacture of a medicament for stimulating humoral and/or T cell responses to said antigen.

5. The recombinant fusion protein of any one of claims 1 or 2, process of claim 3 or use of claim 4 wherein the hsp is a hsp90, hsp70, hsp60 or small hsp family member.

6. The recombinant fusion protein of any one of claims 1 or 2, process of claim 3 or use of claim 4 wherein the hsp is a fungal, viral or eukaryotic stress protein.

7. The recombinant fusion protein of any one of claims 1 or 2, process of claim 3 or use of claim 4 wherein the hsp is a bacterial stress protein.

8. The recombinant fusion protein, process or use of claim 7 wherein the bacterial stress protein is a DnaJ, DnaK, GroES or GroEL family member.

9. The recombinant fusion protein, process or use of claim 7 wherein the bacterial stress protein is a mycobacterial stress protein.

10. The recombinant fusion protein, process or use of claim 9 wherein the mycobacterial stress protein is hsp65.

11. The recombinant fusion protein, process or use of claim 10 wherein the hsp65 is *M. bovis* BCG, *M. tuberculosis* or *M. leprae* hsp65.

12. The recombinant fusion protein, process or use of claim 9 wherein the mycobacterial stress protein is hsp 70.

13. The recombinant fusion protein, process or use of claim 12 wherein the mycobacterial stress protein is *M. tuberculosis* or *M. leprae* hsp70.

14. The recombinant fusion protein of any one of claims 1 or 2, process of claim 3 or use of claim 4 wherein the hsp is a hsp of a parasite.

15. The recombinant fusion protein, process, or use of any one of the preceding claims, wherein the antigen is an antigen of a cancer cell.

16. The recombinant fusion protein, process, or use of any one of claims 1 to 14, wherein the antigen is a viral antigen.

17. The recombinant fusion protein, process or use of claim 16 wherein the viral antigen is an HIV protein.

18. The recombinant fusion protein, process or use of claim 17 wherein the HIV protein is the HIV p24, gag or pol protein.

Patentansprüche

1. Rekombinantes Fusionsprotein, das (a) ein Hitzeschock-Protein (hsp) oder (b) ein Protein, das mit hsp zu zumindest 40% identisch ist, oder (c) einen Teil des hsp oder des Proteins, wobei hsp, Protein oder Teil dazu in der Lage ist, humorale und/oder T-Zell-Reaktionen zu stimulieren und (d) ein Antigen umfasst, zur Verwendung in der Immuntherapie oder in der Prophylaxe.

2. Rekombinantes Fusionsprotein nach Anspruch 1, bei dem *das* Protein mit hsp zu ungefähr 50% identisch ist.

3. Verfahren zur Herstellung eines rekombinanten Fusionsproteins zur Verwendung in der Immuntherapie oder Prophylaxe, wobei das Verfahren die Schritte umfasst, ein wie in einem der Ansprüche 1 oder 2 definiertes hsp, Protein oder Teil durch rekombinante Mittel mit einem Antigen zu verbinden.

4. Verwendung eines rekombinanten Fusionsproteins wie in einem der Ansprüche 1 oder 2 definiert, das durch das Verfahren von Anspruch 3 herstellbar ist, zur Herstellung eines Medikamentes zur Stimulierung von humoralen und/oder T-Zell-Reaktionen auf das Antigen.

5. Rekombinantes Fusionsprotein nach einem der Ansprüche 1 oder 2, Verfahren nach Anspruch 3 oder Verwendung nach Anspruch 4, wobei das hsp hsp90, hsp70, hsp60 oder Mitglied der kleinen hsp-Familie ist.

6. Rekombinantes Fusionsprotein nach einem der Ansprüche 1 oder 2, Verfahren nach Anspruch 3 oder Verwendung nach Anspruch 4, wobei das hsp ein Pilz-, virales oder eukaryotisches Stressprotein ist.

7. Rekombinantes Fusionsprotein nach einem der Ansprüche 1 oder 2, Verfahren nach Anspruch 3 oder Verwendung nach Anspruch 4, wobei das hsp ein bakterielles Stressprotein ist.

8. Rekombinantes Fusionsprotein, Verfahren oder Verwendung nach Anspruch 7, wobei das bakterielle Stressprotein DnaJ, Dnak, GroES oder GroEL- Familienmitglied ist.

9. Rekombinantes Fusionsprotein, Verfahren oder Verwendung nach Anspruch 7, wobei das bakterielle Stressprotein ein mykobakterielles Protein ist.

10. Rekombinantes Fusionsprotein, Verfahren oder Verwendung nach Anspruch 9, wobei das mykobakterielle Stressprotein hsp65 ist.

11. Rekombinantes Fusionsprotein, Verfahren oder Verwendung nach Anspruch 10, wobei das hsp65 *M. bovis* MCG, *M. tuberculosis* oder *M. leprae* hsp65 ist.

12. Rekombinantes Fusionsprotein, Verfahren oder Verwendung nach Anspruch 9, wobei das mykobakterielle Stressprotein hsp70 ist.

13. Rekombinantes Fusionsprotein, Verfahren oder Verwendung nach Anspruch 12, wobei das mykobakterielle Stressprotein *M. tuberculosis* oder *M. leprae* hsp70 ist.

14. Rekombinantes Fusionsprotein nach einem der Ansprüche 1 oder 2, Verfahren nach Anspruch 3 oder Verwendung nach Anspruch 4, wobei das hsp ein hsp eines Parasiten ist.

15. Rekombinantes Fusionsprotein, Verfahren oder Verwendung nach einem der vorhergehenden Ansprüche, wobei das Antigen ein Antigen einer Krebszelle ist.

16. Rekombinantes Fusionsprotein, Verfahren oder Verwendung nach einem der Ansprüche 1-14, wobei das Antigen ein virales Antigen ist.

17. Rekombinantes Fusionsprotein, Verfahren oder Verwendung nach Anspruch 16, wobei das virale Antigen ein HIV-Protein ist.

18. Rekombinantes Fusionsprotein, Verfahren oder Verwendung nach Anspruch 17, wobei das HIV-Protein das HIV p24, gag- oder pol-Protein ist.

Revendications

1. Une protéine de fusion recombinante comprenant (a) une protéine de choc thermique (hsp), ou (b) une protéine étant au moins identique à 40% à cette hsp, ou (c) une partie de cette hsp ou protéine, laquelle hsp, protéine ou partie est capable de stimuler des réponses humorales et/ou par des cellules T et (d) un antigène, à utiliser dans la thérapie immunitaire ou la prophylaxie.

2. Protéine de fusion recombinante selon la revendication 1, dans laquelle la protéine est approximativement identique à 50% à hsp.

3. Procédé pour produire une protéine de fusion recombinante à utiliser dans une thérapie immunitaire ou une prophylaxie, le procédé comprenant l'étape de joindre une hsp, une protéine ou une partie, telle que définie dans l'une quelconque des revendications 1 ou 2, à un antigène par un moyen recombinant.

4. Utilisation d'une protéine de fusion recombinante telle que définie dans l'une quelconque des revendications 1 ou 2 réalisable par le procédé de la revendication 3 pour la fabrication d'un médicament pour stimuler les réponses humorales et/ou par des cellules T à cet antigène.

5. La protéine de fusion recombinante selon l'une quelconque des revendications 1 ou 2, le procédé selon la revendication 3 ou utilisation selon la revendication 4 dans laquelle hsp est une hsp90, hsp70, hsp60 ou un petit membre de la famille hsp.

6. Protéine de fusion recombinante selon l'une quelconque des revendications 1 ou 2, procédé selon la revendication 3 ou utilisation selon la revendication 4 dans laquelle hsp est une protéine de stress issue d'un champignon, d'un virus ou d'un eucaryote.

7. La protéine de fusion recombinante selon L'une quelconque des revendications 1 ou 2, le procédé selon la revendication 3 ou l'utilisation selon la revendication 4 dans laquelle hsp est une protéine de stress issue d'une bactérie.

8. Protéine de fusion recombinante, procédé ou utilisation selon la revendication 7 dans laquelle la protéine de stress issue d'une bactérie est un membre de la famille d'un AdnJ, AdnK, GroES ou GroEL.

9. Protéine de fusion recombinante, procédé ou utilisation selon la revendication 7 dans laquelle la protéine de stress issue d'une bactérie est une protéine de stress issue d'une mycobactérie.

10. Protéine de fusion recombinante, procédé ou utilisation selon la revendication 9 dans laquelle la protéine de stress issue d'une mycobactérie est une hsp65.

11. Protéine de fusion recombinante, procédé ou utilisation selon la revendication 10 dans laquelle hsp65 est M. bovis BCG, M. tuberculosis ou M. leprae hsp65.

12. Protéine de fusion recombinante, procédé ou utilisation selon la revendication 9 dans laquelle la protéine de stress

issue d'une mycobactérie est hsp70.

13. Protéine de fusion recombinante, procédé ou utilisation selon la revendication 12 dans laquelle la protéine de stress issue d'une mycobactérie est M. tuberculosis ou M. leprae hsp70.

14. Protéine de fusion recombinante selon l'une quelconque des revendications 1 ou 2, procédé selon la revendication 3 ou utilisation selon la revendication 4 dans laquelle hsp est une hsp d'un parasite.

15. Protéine de fusion recombinante, procédé, ou utilisation selon l'une quelconque des revendications précédentes, dans laquelle l'antigène est un antigène d'une cellule cancéreuse.

16. Protéine de fusion recombinante, procédé, ou utilisation selon l'une quelconque des revendications 1 à 14, dans laquelle l'antigène est un antigène viral.

17. Protéine de fusion recombinante, procédé ou utilisation selon la revendication 16 dans laquelle l'antigène viral est une protéine du VIH.

18. Protéine de fusion recombinante, procédé ou utilisation selon la revendication 17 dans laquelle la protéine du VIH est la protéine p24, gag ou pol du VIH.

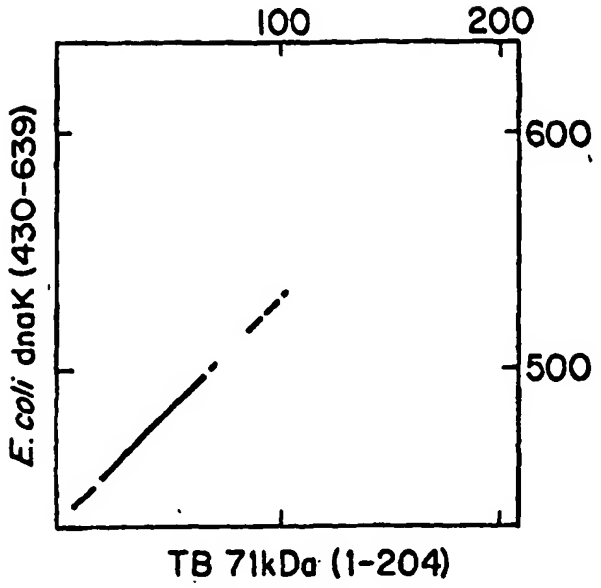

100
200
600
500
E. coli dnaK (430-639)
TB 71kDa (1-204)

Fig. 1A

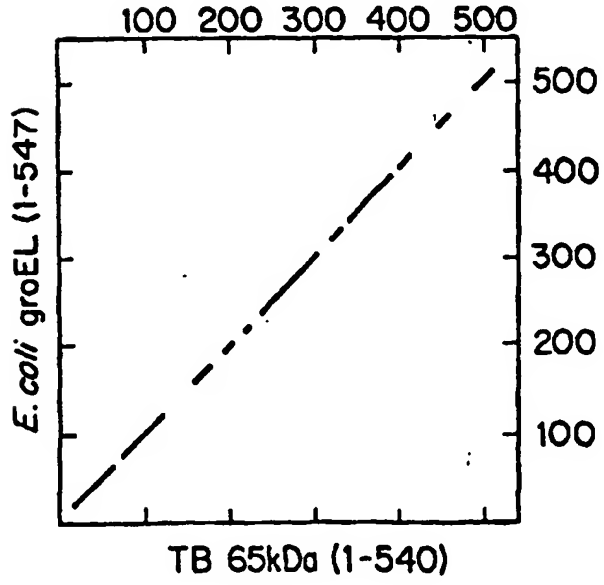

100 200 300 400 500
500
400
300
200
100
E. coli groEL (1-547)
TB 65kDa (1-540)

Fig. 1B

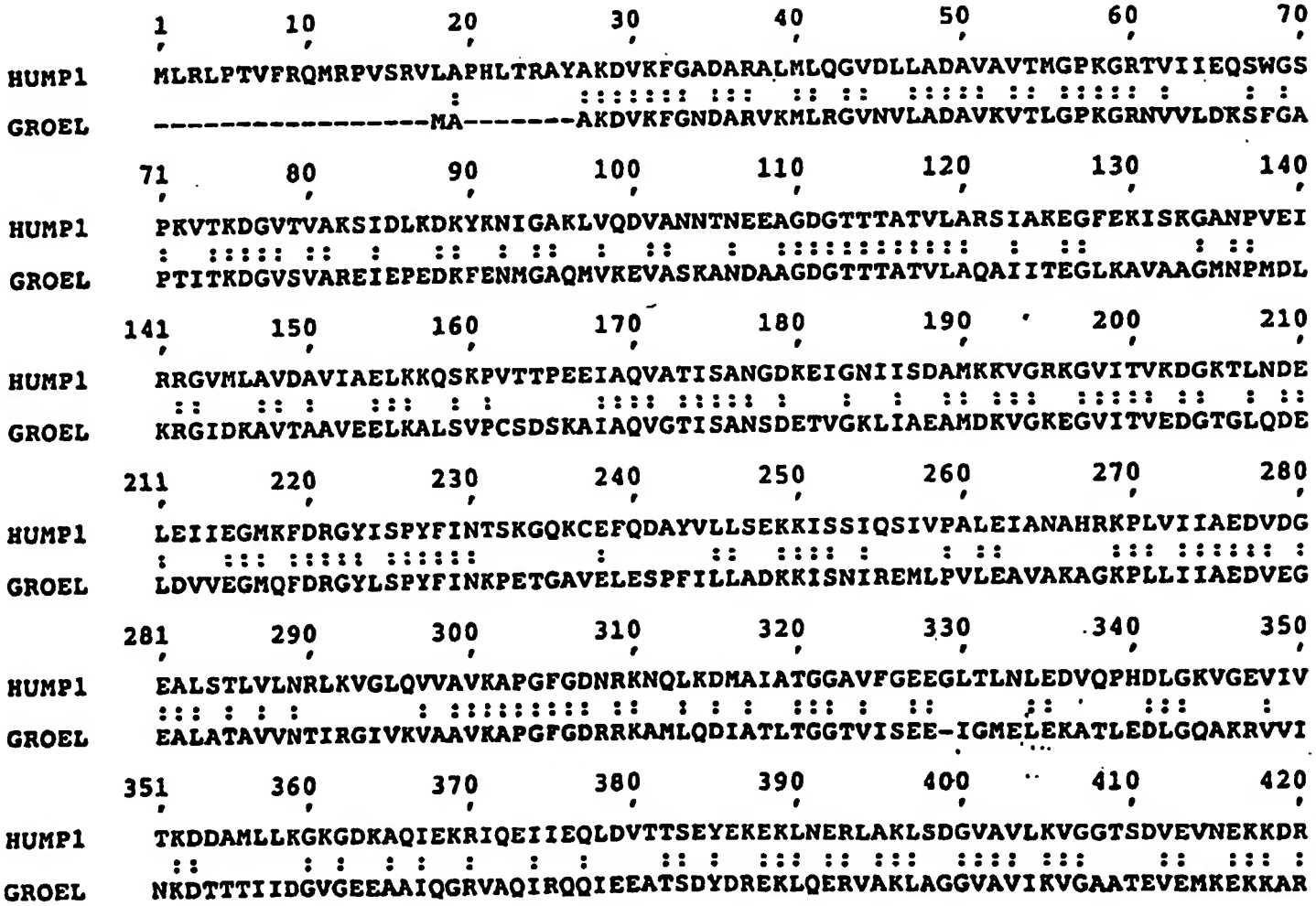


FIGURE 2

EP 0 700 445 B1

```
        421       430       440       450       460       470       480       490
HUMP1     VTDALNATRAAVEEGIVLGGGCALLRCIPALDSLTPANEDQKIGIEIIKRTLKIPAMTIAKNAGVEGSLI
          : ::: :::::::::: : ::: :: :    :  :  ::::         :    :   :  : : : :
GROEL     VEDALHATRAAVEEGVVAGGGVALIRVASKLADLRGQNEDQNVVSSSL-RAMEAPLRQIVLNCGEEPSVV

        491       500       510       520       530       540       550       560
HUMP1     VEKIMQSSSEVGYDAMAGDFVNMVEKGIIDPTKVVRTALLDAAGVASLLTTAEVVVTEIPKEEKDPGMGA
                  :: :      ::   :: :::::: : ::  :: :: :  : : :  ::  ::       ::
GROEL     ANTVKGGDGNYGYNAATEEYGNMIDMGILDPTKVTRSALQYAASVAGLMITTECMVTDLPKND-AADLGA

        561       570
HUMP1     MGGMGG--GMGGGMF
           :::::  :::: :
GROEL     AGGMGGMGGMGGMM-

Total score = 4667, 5 breaks
  276 identities out of 545 possible matches between residues

25 random runs
Alignment score =   65.34 SD   Standard deviation =   18.94   Mean = 3429.48
```

FIGURE 2 (continued)

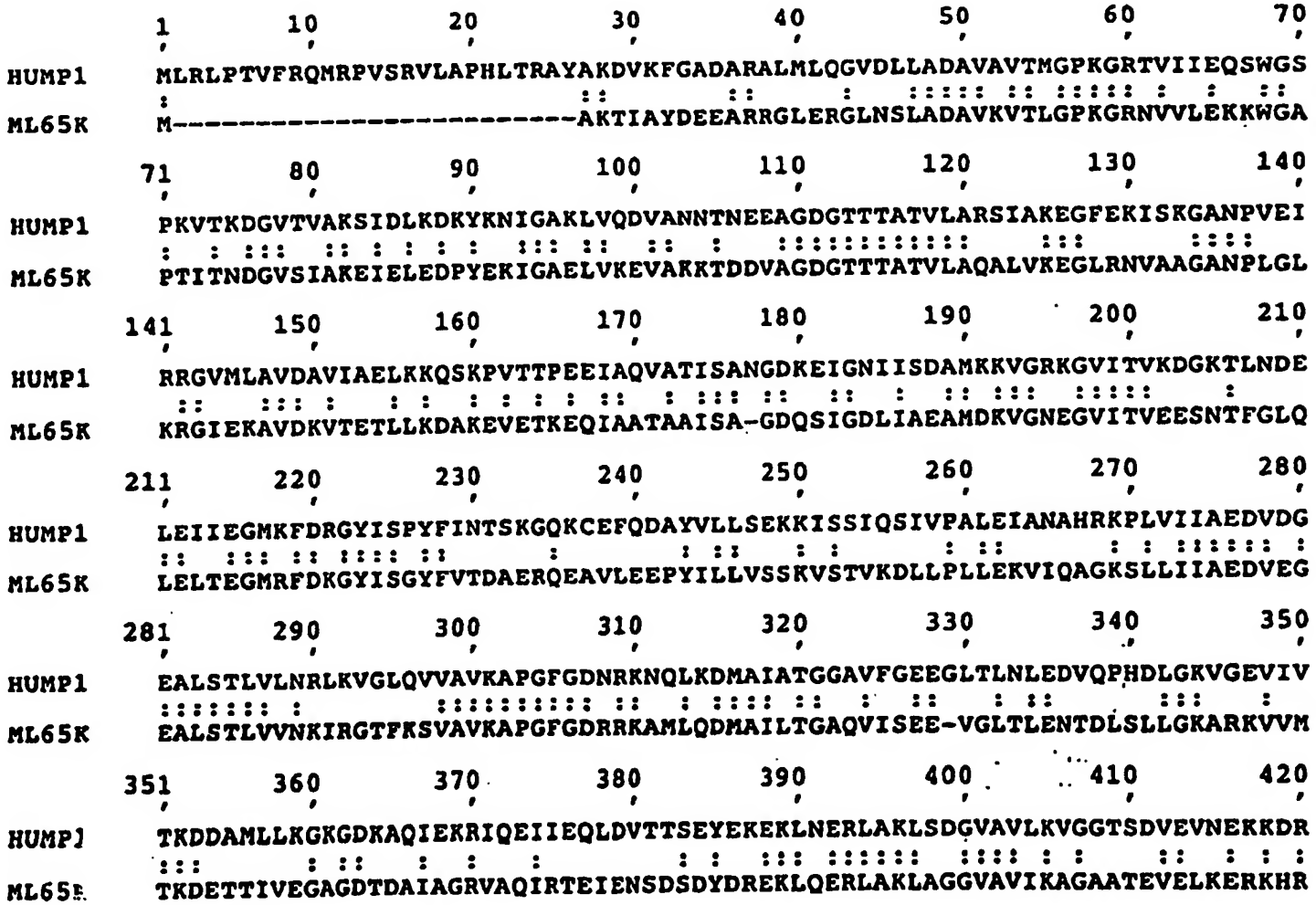


FIGURE 3

EP 0 700 445 B1

```
        421       430       440       450       460       470       480       490
HUMP1     VTDALNATRAAVEEGIVLGGGCALLRCIPALDSLTPANEDQKIGIEIIKRTLKIPAMTIAKNAGVEGSLI
ML65K     IEDAVRNAKAAVEEGIVAGGGVTLLQAAPALDKLKLTGDEAT-GANIVKVALEAPLKQIAFNSGMEPGVV

        491       500       510       520       530       540       550       560
HUMP1     VEKIMQSSSEVGYDAMAGDFVNMVEKGIIDPTKVVRTALLDAAGVASLLTTAEVVVTEIPKEEKDPGMGA
ML65K     AEKVRNLSVGHGLNAATGEYEDLLKAGVADPVKVTRSALQNAASIAGLFTT-EAVVADKPEKTAAPASDP

        561       570
HUMP1     MGGMGGGMGGGMF
ML65K     TGGMGG-MD---F
```

Total score = 4552, 7 breaks
255 identities out of 540 possible matches between residues

25 random runs
Alignment score = 47.73 SD Standard deviation = 23.86 Mean = 3413.16

FIGURE 3 (continued)

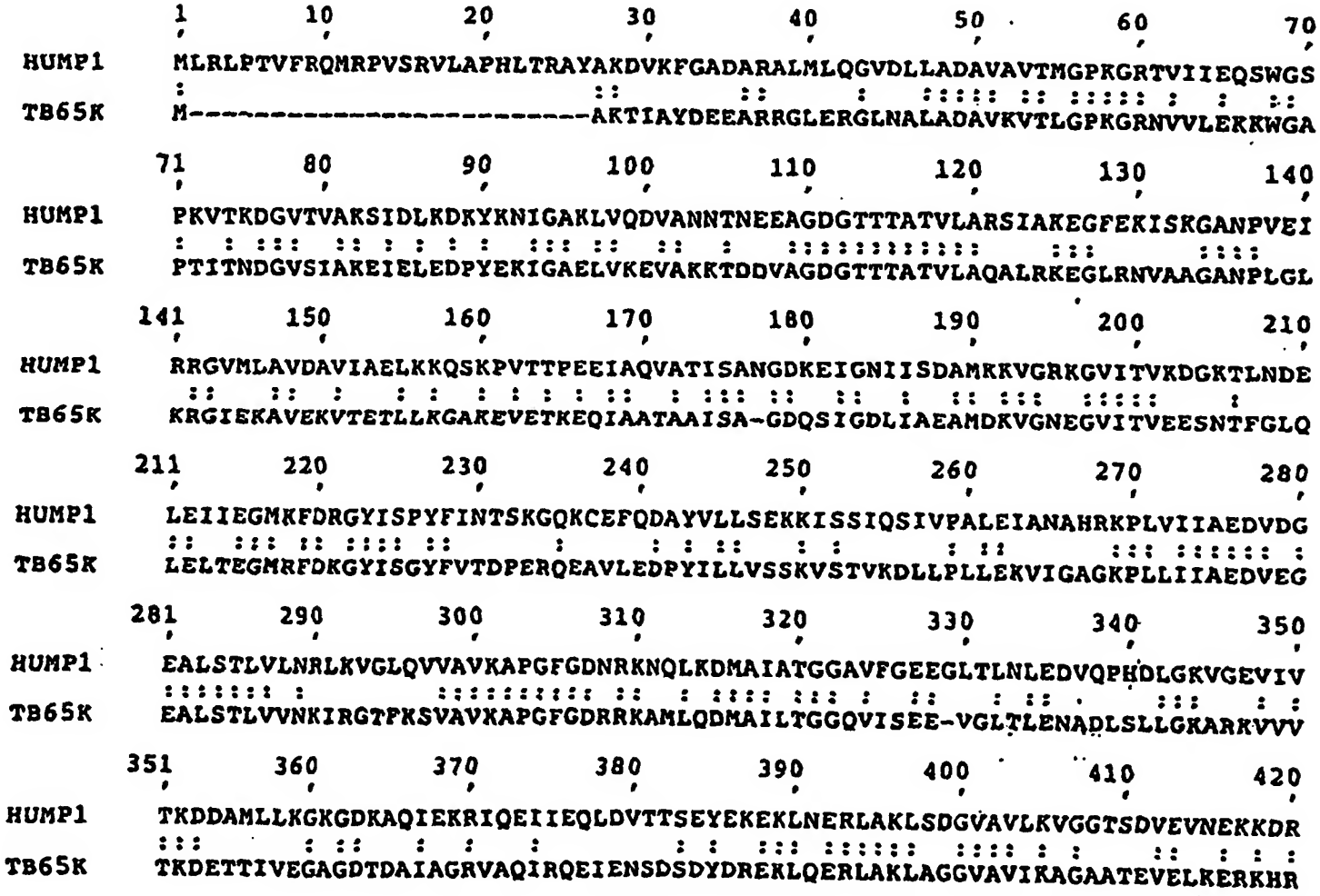
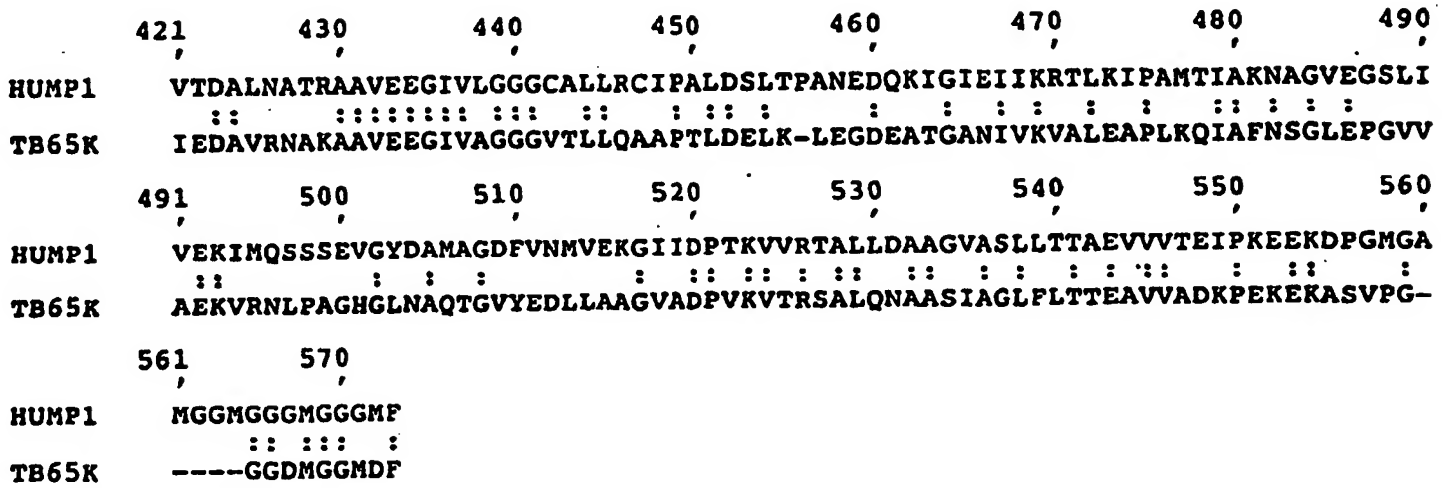

1 10 20 30 40 50 60 70
HUMP1 MLRLPTVFRQMRPVSRVLAPHLTRAYAKDVKFGADARALMLQGVDLLADAVAVTMGPKGRTVIIEQSWGS
TB65K M----------AKTIAYDEEARRGLERGLNALADAVKVTLGPKGRNVVLEKKWGA
71 80 90 100 110 120 130 140
HUMP1 PKVTKDGVTVAKSIDLKDKYKNIGAKLVQDVANNTNEEAGDGTTTATVLARSIAKEGFEKISKGANPVEI
TB65K PTITNDGVSIAKEIELEDPYEKIGAELVKEVAKKTDDVAGDGTTTATVLAQALRKEGLRNVAAGANPLGL
141 150 160 170 180 190 200 210
HUMP1 RRGVMLAVDAVIAELKKQSKPVTTPEEIAQVATISANGDKEIGNIISDAMKKVGRKGVITVKDGKTLNDE
TB65K KRGIEKAVEKVTETLLKGAKEVETKEQIAATAAISA-GDQSIGDLIAEAMDKVGNEGVITVEESNTFGLQ
211 220 230 240 250 260 270 280
HUMP1 LEIIEGMKFDRGYISPYFINTSKGQKCEFQDAYVLLSEKKISSIQSIVPALEIANAHRKPLVIIAEDVDG
TB65K LELTEGMRFDKGYISGYFVTDPERQEAVLEDPYILLVSSKVSTVKDLLPLLEKVIGAGKPLLIIAEDVEG
281 290 300 310 320 330 340 350
HUMP1 EALSTLVLNRLKVGLQVVAVKAPGFGDNRKNQLKDMAIATGGAVFGEEGLTLNLEDVQPHDLGKVGEVIV
TB65K EALSTLVVNKIRGTFKSVAVKAPGFGDRRKAMLQDMAILTGGQVISEE-VGLTLENADLSLLGKARKVVV
351 360 370 380 390 400 410 420
HUMP1 TKDDAMLLKGKGDKAQIEKRIQEIIEQLDVTTSEYEKEKLNERLAKLSDGVAVLKVGGTSDVEVNEKKDR
TB65K TKDETTIVEGAGDTDAIAGRVAQIRQEIENSDSDYDREKLQERLAKLAGGVAVIKAGAATEVELKERKHR

FIGURE 4



Total score = 4560, 5 breaks
257 identities out of 540 possible matches between residues

25 random runs
Alignment score = 49.36 SD Standard deviation = 23.23 Mean = 3413.16

FIGURE 4 (continued)

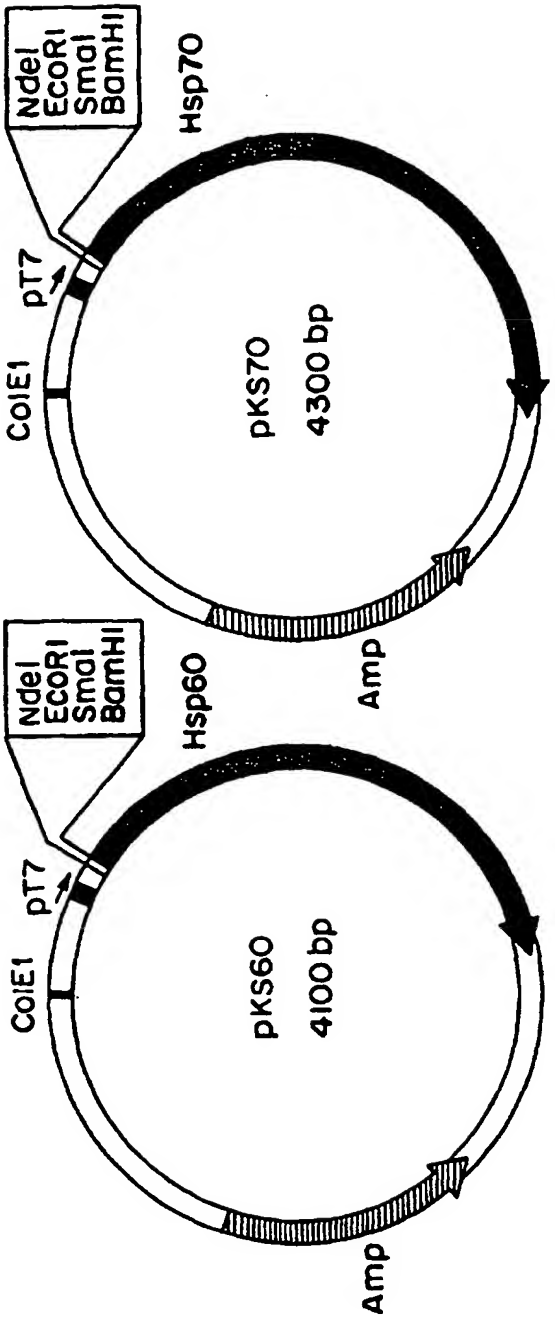

NdeI
EcoRI
SmaI
BamHI
Hsp70
pT7
ColE1
pKS70
4300 bp
Amp
NdeI
EcoRI
SmaI
BamHI
Hsp60
pT7
ColE1
pKS60
4100 bp
Amp

FIG. 5

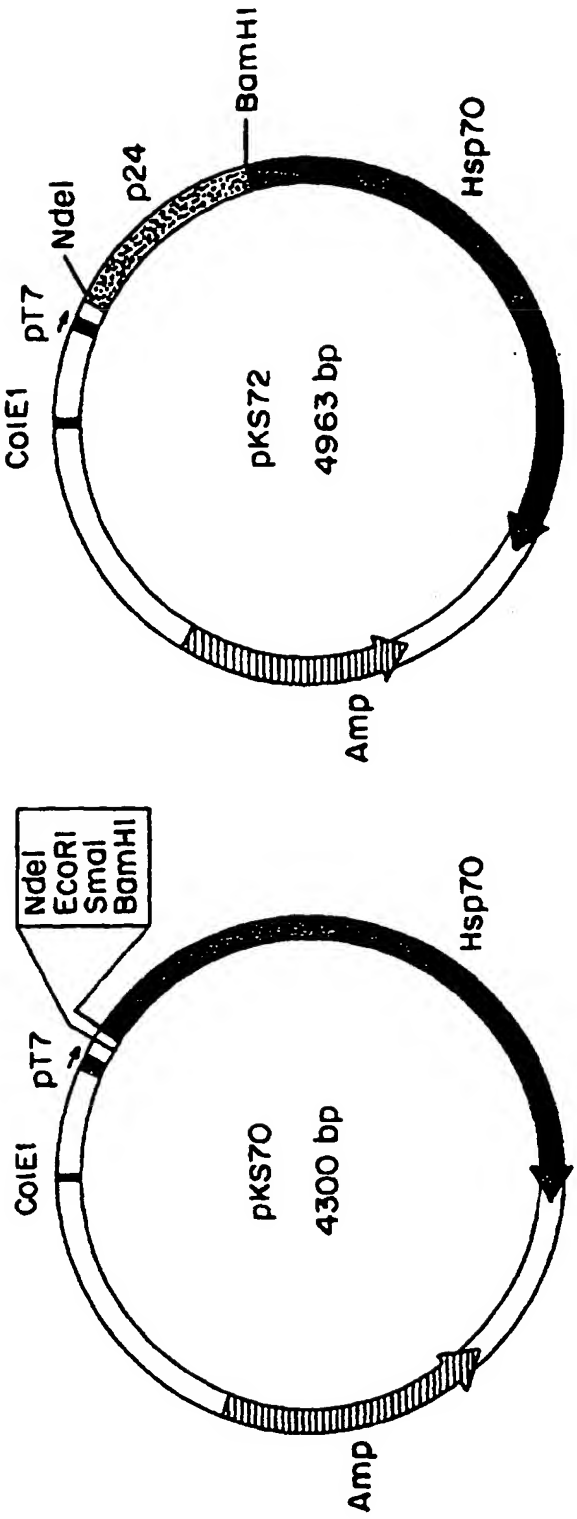

BamHI
Hsp70
p24
NdeI
pT7
ColE1
pKS72
4963 bp
Amp
NdeI
EcoRI
SmaI
BamHI
Hsp70
pT7
ColE1
pKS70
4300 bp
Amp

FIG. 6

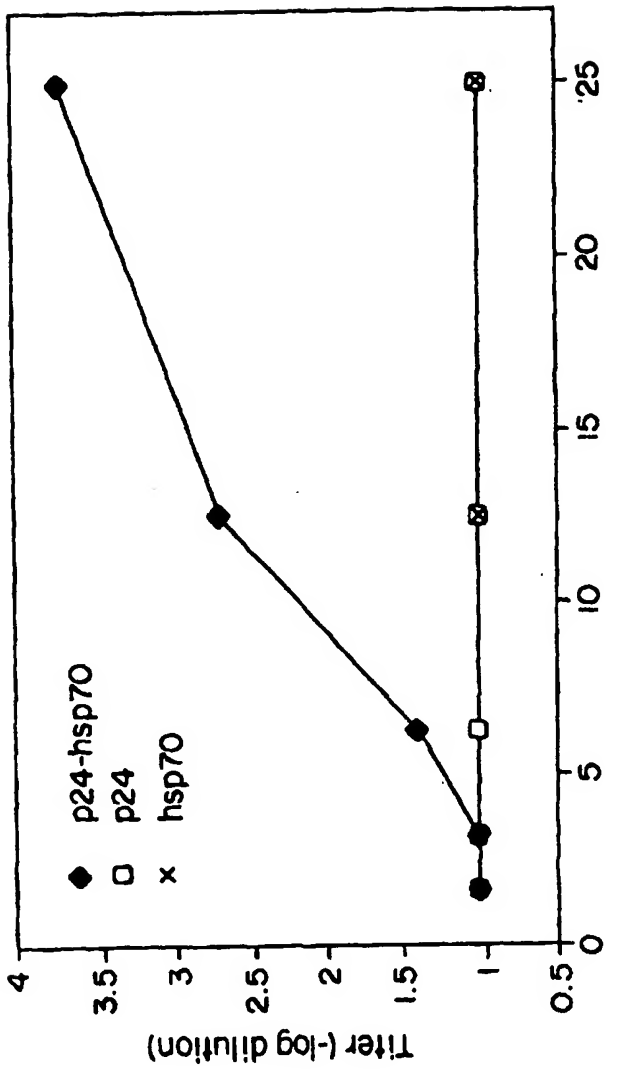

Titer (-log dilution)
4
3.5
3
2.5
2
1.5
1
0.5
0
5
10
15
20
25
pmoles
p24-hsp70
p24
hsp70

FIG. 7